TRACHEAL GAS DYNAMICS IN PUPAE OF THE CECROPIA SILKWORM

JOHN W. KANWISHER [1]

The Biological Laboratories, Harvard University, Cambridge, Massachusetts

Once a Cecropia larva pupates and spins a cocoon in late summer it is destined to neither eat or drink for the rest of its life—some 8 months. It is only with the emergence of the next generation caterpillar from the egg the following spring that these activities are resumed. Strict water conservation in the pupa over this long period is obviously an urgent necessity if the insect is to avoid dehydration. The remarkable water impermeability of the insect cuticle has long been known. But the problem remains of how the insect can breathe without excessive loss of water.

The tracheal system is the pathway for oxygen and carbon dioxide exchange between the metabolizing tissues and the external environment. Because they provide such ready access for respiratory gases, the tracheal tubes are also unavoidably an easy path for water vapor loss. This, in effect, represents a shunt around the impermeable cuticle which seems calculated to dry the insect from the inside out. The insects' answer has been to open the tracheae only intermittently to the outside air; the rest of the time they are effectively sealed against the outflow of water vapor by spiracular valves (Buck, 1962).

Ideally, the spiracles should open only frequently enough to avoid anoxia to the tissues. Any additional opening represents an unwarranted water loss. Operationally, a barely sufficient ventilation is ingeniously insured by placing the spiracular valves under the control of the gases within the tracheae (Wigglesworth, 1935; Schneiderman and Williams, 1955). A combination of low pO_2 and high pCO_2 triggers the valves to open for a brief period of rapid gas exchange. The tracheae are then nearly closed off from the air for long periods.

This intermittent or "burst respiration" has received much attention (Beckel, 1958; recent review by Buck, 1962) ; despite this fact, much remains to be understood about the dynamics of how it works. A new analysis will be attempted here. Our chief concern will be with experimentally verifiable rather than logically reasonable hypotheses.

METHODS AND RESULTS

1. *Thermal conductivity recording*

a. CO_2 production

Study of CO_2 bursts requires that one determine the timetable for a given animal and thus be able to predict when the next burst will occur. Punt's (1944) diaferometer allowed continuous CO_2 output recording but its baseline drifted. Most

[1] Present address: Woods Hole Oceanographic Institution, Woods Hole, Massachusetts 02543.

recent work has been done manometrically. The laboriousness of this approach is evident when one contemplates reading a microrespirometer every 15 minutes for several days. I have developed a continuous CO_2 recorder, or diaferometer, based on the thermal conductivity changes which CO_2 produces in air. It measures the change in resistance of a heated thermistor which is placed in the gas stream.

Oxygen and nitrogen have nearly the same thermal conductivity. That for carbon dioxide is higher, while the value for water vapor is lower. If an insect withdraws oxygen in a gas flow respirometer, there is very little change in the conductivity of the efferent gas. But if it introduces carbon dioxide or water, there can be a detectable change.

The equilibrium temperature of a thermistor with a constant electrical power input is a function of the thermal conductivity of the gas mixture surrounding it. In a high conductivity environment the thermistor can lose its heat more readily and comes to a lower temperature. With low conductivity the opposite is the case.

Two thermistors were used in a balanced bridge circuit, one being placed in the stream of gas supplied to the experimental object and the other in the efferent stream. One can readily make this arrangement record 0% to 1% CO_2 in air. It is interesting to calculate what this means in temperature difference of the thermistors. The applied electrical power heats them to about 150° C. in air. The addition of 1% CO_2 changes the temperature of one of them only 0.1°. But this is enough to provide 10 mv unbalance in the bridge and drive a recorder to full scale. With a 0.01% CO_2 sample the thermistors differ in temperature by only 0.001°. It is obvious from this that voltages and environmental temperatures of the thermistors must be carefully regulated. New solid state techniques simplify these tasks. Water vapor must also be removed after the gas stream passes the pupa and before it reaches the second thermistor.

One can automatically record every burst during weeks of metabolism. It is possible to get data from several insects at once by use of a multiple channel recorder. Animals can be quickly tested to determine if they have started developmental metabolism. Irregularities in burst period from pupa to pupa are readily apparent. Spiracular valve malfunctions show up clearly. At a chart speed of 1 inch/hour one can see the entire three-week developmental sequence of a Cecropia on a 50-foot record. Some records are presented in Figure 1.

b. Water vapor loss

The "burst mechanism" is probably of value to the insect primarily for water conservation (Beckel, 1958). Thermal conductivity can also be used to record continuously the water vapor coming from a pupa. Two thermistors are placed in the efferent air stream before and after a drying chamber in the gas stream. The change is then due only to the water vapor. With two bridges and three thermistors one can simultaneously record CO_2 and H_2O output.

Water vapor varies the thermal conductivity in the opposite direction from CO_2. The difference of its thermal conductivity from O_2 and N_2 is only half as great as CO_2. Thus the electrical signal from the bridge will be of opposite polarity and of only half the magnitude. Superimposed records of the two gases both separately and combined are shown in Figure 2.

c. Other continuous methods

An infrared analyzer also can be used to record CO_2 output. Since this technique works in the parts-per-million concentration range, it is valuable for looking at interburst CO_2 output. Such an analyzer is 2000 times more sensitive to CO_2 than to H_2O, so drying of the gas stream is not important. By recirculating the air in a closed circuit loop I was able to show the gradual increase in CO_2 concentration that results from the interburst CO_2 output of the pupa.

When the spiracles open during a burst, the rapid water vapor loss cools the pupa about 0.1° C. This cyclical cooling can be recorded by a thermistor placed on the cuticle under a tuft of cotton. The other thermistor of a bridge is also placed in the animal chamber so that ambient temperature changes produce no
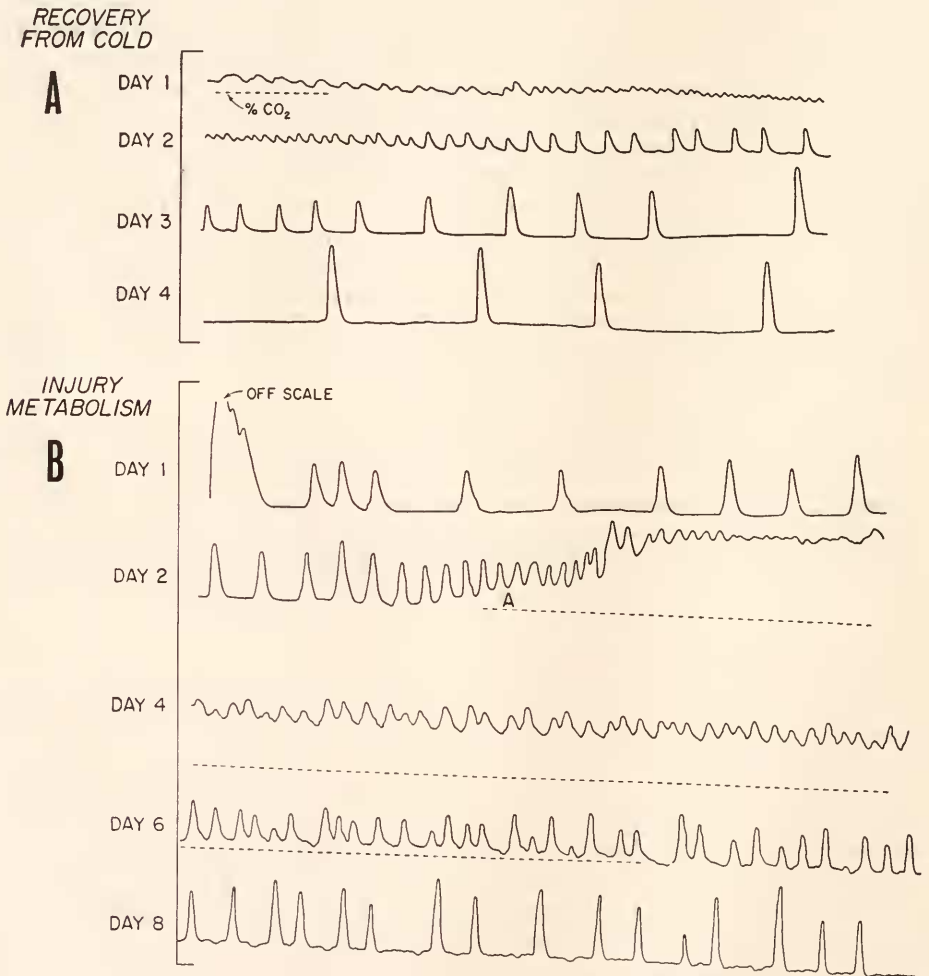

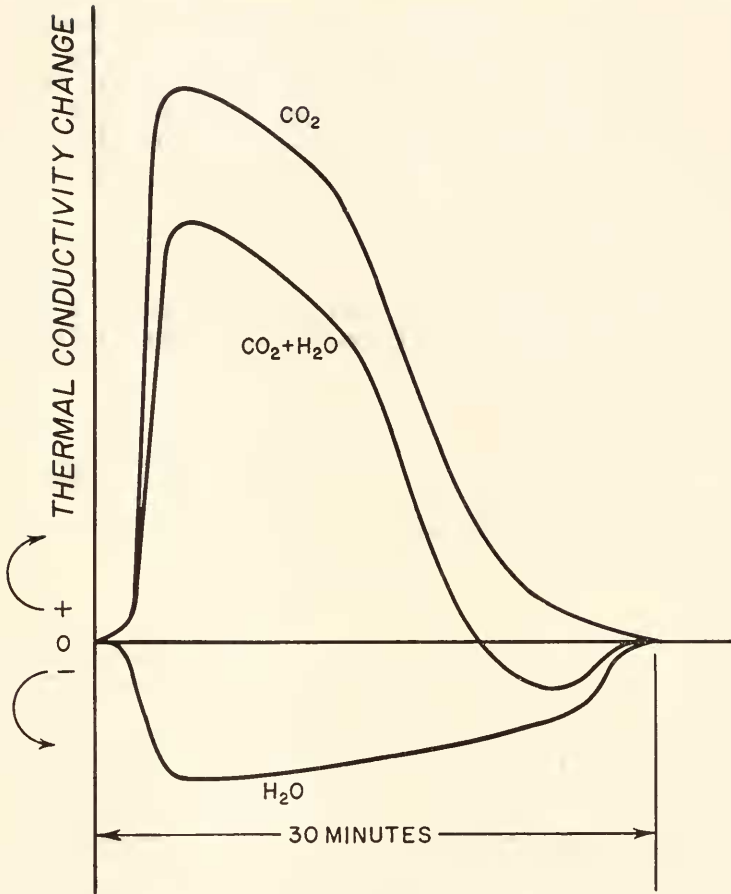FIGURE 1. CO_2 output recording by thermal conductivity of insect pupa.

FIGURE 2. Thermal conductivity recording of CO_2, water vapor, and both combined, for a single burst.

output. This is the most simple arrangement if one only desires the time of occurrence of the bursts.

2. Tracheal-atmospheric gas exchange

A pupa consumes O_2 from the air and excretes CO_2. The CO_2 is known to come out partly in bursts. The time course of O_2 entry has not been determined directly. Manometry shows that O_2 is used internally at a constant rate. But the tracheal gas volume is a reservoir that the tissues can draw on without using outside air.

The flux of CO_2 and O_2 in and out of the spiracles has been measured simultaneously here by direct gas analysis. A pupa was suspended in a chamber of 100 to 200 ml. volume in which the air was kept at a slight positive pressure (about 3 cm. Hg). A stockcock and nipple on the top of the chamber allowed samples

to be pipetted directly to a Scholander 0.5-cc. analyzer. Readings from this analyzer (Scholander, 1947) were accurate to 0.015% for CO_2 and to 0.02% for O_2. Serial readings gave the time course of the gas mixture in the animal chamber. Gradual decrease in volume as samples were withdrawn was corrected by calculation. Although this is a laborious technique, it provides definitive data on the gases passing in and out of the animal. An example of these data is shown in Figure 3.

3. Tracheal volume and gas composition

Tracheal volume was measured by pupal compressibility in the same way as had been applied to fish swim bladders (Kanwisher and Ebeling, 1957). The pupa
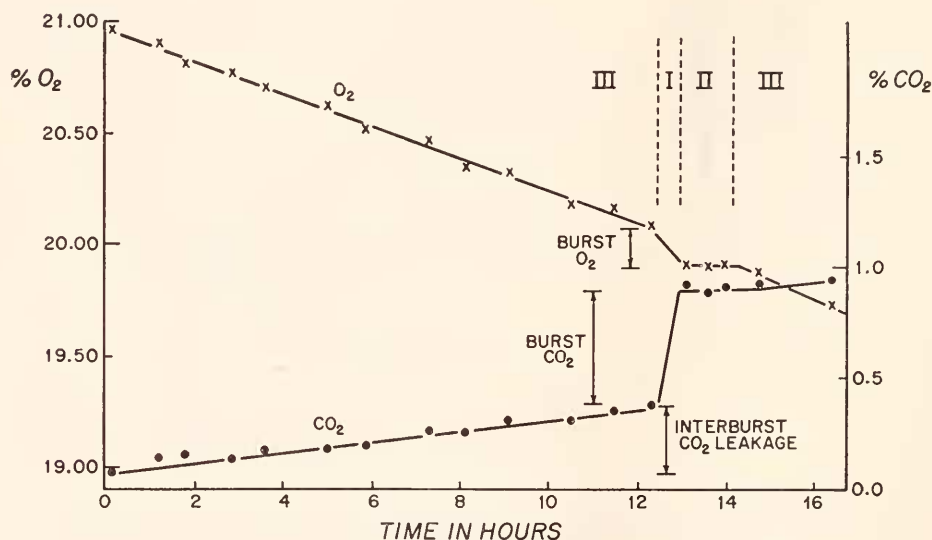

FIGURE 3. Time change of O_2 and CO_2 concentration in a sealed chamber containing a single pupa.

was sealed in a rigid water-filled glass chamber connected to a tuberculin syringe. A trace of detergent was added to the water to eliminate surface bubbles. A small closed-end manometer in the chamber indicated pressure. The volume increments necessary to change the pressure were used to compute the volume according to Boyle's law. The pleated cuticle structure was assumed to exert no mechanical resistance to compression. The tracheal volume was about 5% of that of the entire body (average of 10 readings). For a 5-gram pupa this amounts to 250 mm.³. This method gives less spread (4% to 7%) than that of Buck and Keister (1958).

Levy and Schneiderman (1958) have provided the only figures on tracheal gas composition. After a ventilating burst, pO_2 and pCO_2 are, respectively, about 18% and 3%. During the initial interburst period pO_2 drops rapidly to 5% and pCO_2 increases slightly to 4%. Over the several hours until the next burst, pO_2 remains constant and pCO_2 rises to about 6%.

The tracheal gas pO_2 figures were checked by rapid vacuum extraction of the tracheal gas. A pupa in a syringe filled with concentrated citrate solution (to reduce gas solubility) was subjected to a rapid expansion. Gas visibly flowed out of some of the spiracles. This was quickly collected in an adjoining 1-ml. syringe and analyzed (Scholander *et al.*, 1955). Pupae which had just completed a burst gave off tracheal gas with 15% to 19% O_2. At the end of the interburst period the gas contained 5% to 8% O_2. The CO_2 fraction varied from 2% to 8%. Higher values were at the end of the burst period. The greater mobility of CO_2 and its buffering by body fluids make the values for this gas less reliable.

This tracheal gas analysis method required sacrificing the animal so that sequential values on a single individual were not possible. However, the values represent a fair sampling of the entire tracheal gas system since sometimes as much as 150 mm.³ were obtained by this vacuum purging technique. It appears that the measurements of Levy and Schneiderman on gas samples from a cannulated spiracle are reasonably representative of the entire tracheal system. Buck (1962) surmised this homogeneity by reasoning that the main diffusive barrier is within the tissues rather than in the trachea. Buck and Keister (1958) also obtained figures for pCO_2 which are in general agreement.

<center>DISCUSSION</center>

1. Spiracular gas flux

Gas exchange between the air and the tissue takes place through the tracheal reservoir. Oxygen is in one-way transit from the air to the tissues. Warburg records show that it enters the tissues at a constant rate. Carbon dioxide moves discontinuously in the opposite direction because of bicarbonate buffering and intermittent tracheal ventilation. Nitrogen is an inert filler which passes in and out of the trachea through the spiracles to make up the volume deficit produced by CO_2 buffering and also by any departure of the R.Q. from unity. The volume of the tracheal system is assumed to remain constant.

To analyze this gas exchange system requires the coordination of the various types of data mentioned. For this purpose it is convenient to consider a burst sequence as consisting of three phases. Stage I, when the valves are open and gas exchange is rapid, lasts 15 to 20 minutes. Stage II covers roughly the first hour of the interburst period; during this time the tracheal pO_2 drops rapidly and pCO_2 increases slightly. The pN_2 increases to make up the difference. Stage III extends for the rest of the interburst period: the pO_2 remains low and constant, pCO_2 increases slightly, and pN_2 drops the same amount to make up the difference. The tracheal gas compositions (from Levy and Schneiderman, 1958) at the end of these phases are given in Table I. The changes in tracheal gas composition are also included.

From these figures we can immediately compute the flux of N_2 for a hypothetical 5-gm. pupa with a tracheal volume of 250 mm.³. During the burst (stage I), 27 mm.³ of N_2 flow out through the spiracles. Strictly speaking, about another 20% should be added in allowance for N_2 dissolved in the tissues. In stage II 30 mm.³ flow back in. During stage III the difference of 3 mm.³ must flow out.

Oxygen uptake presents more difficulties. We assume that it enters the tissues

TABLE I

Tracheal gas composition (in atmospheres)

Change during I	End of I	Change during II	End of II	Change during III	End of III
O_2 + 0.13	0.18	−0.13	0.05	0	0.05
CO_2 − 0.03	0.03	+0.01	0.04	+0.02	0.06
N_2 − 0.11	0.79	+0.12	0.91	−0.02	0.89

at a steady rate. With a reasonable diapause metabolic rate of 20 mm.³ O_2/gm./hr. the uptake for the whole pupa is 100 mm.³/hr. During a 30-minute burst 50 mm.³ of O_2 must thus enter the tissues. At the same time the tracheal gas increases from 5% to 18% O_2 which requires an additional 32 mm.³ O_2. The sum of these, 82 mm.³, must flow in through the spiracles while they are wide open for the burst (stage I).

During the hour of stage II, 100 mm.³ flows into the tissues, of which 32 mm.³ comes from change in the tracheal gas composition. The rest must come from outside. But the chamber air shows little change in composition during this time. This would be true if air rather than O_2 alone were entering the spiracles, as Buck proposes. Eventually however, the N_2 presents a disposal problem and so the pO_2 falls due to N_2 dilution. When there is only 5% O_2 left, the valves open slightly or flutter (Schneiderman, 1956). This marks the beginning of stage III during which O_2 enters by pore diffusion driven by a partial pressure difference of $0.21 - 0.05 = 0.16$ atmosphere. For the rest of the interburst period the flux of O_2 through the spiracles equals that into the tissues, and tracheal pO_2 is constant. At the next burst O_2 surges in through the open spiracles as the cycle begins again.

The changes in tracheal pCO_2 are small (3% to 6%), so the flux of CO_2 from the tissues into the tracheae must at all times be nearly equal to that out through the spiracles. Consider the case of a metabolic production of 70 mm.³/hr. (R.Q. = 0.7) and a half-hour burst. During this time tracheal gas change accounts for 7 mm.³ flowing out. But in a pupa with a 15-hour period the CO_2 flux from the tissues during this time may be 100 times this or 700 mm.³. Tracheal gas chamber buffering is clearly negligible. Most of this CO_2 outflow during such a burst must have been chemically combined in the tissues.

In stage II, negligible CO_2 is released (0.5-cc. and infrared analyzers). This is compatible with the inference from O_2 that air is flowing in during this time under a difference in total pressure. Out diffusion against this stream is apparently ineffective. A small portion of the CO_2 produced flows from the tissues into the tracheae. But bicarbonate buffering keeps most of it in solution.

If O_2 diffuses in during stage III, we should expect CO_2 to pass in the opposite direction. During this period there is an average Δ pCO_2 of 5% across the spiracular valve, or about one-third that of pO_2. Since the two gases have nearly the same diffusibility there should be one-third the flux of CO_2 that there is of O_2. This is found in both 0.5-cc. and infrared measurements. It probably matters little that the CO_2 is diffusing out against a greater counter-current of diffusing O_2.

This relatively large interburst release (350 mm.^{3} in our hypothetical case) is spread over such a long time that it is hard to see in manometric records where measurements of CO_2 and O_2 are performed in separate experiments. It also rarely shows up clearly on the thermal conductivity apparatus. This can be expected since the rate of release is only about 1% of the peak value during the burst (17 as compared to 1400 mm.^{3} CO_2/hr.). So, during this time we can say that one-third of the CO_2 produced is released to the tracheae and mostly diffuses out through the spiracles. The two-thirds remainder is buffered in the blood. This is what Buck (1958) had theorized.

In this idealized treatment the time curves for O_2 and CO_2 in a sealed chamber are shown in Figure 3. The interburst CO_2 release shows an increasing slope due to the climb from 4% to 6% in the tracheal gas. Some actual measurements are also given. They show reasonable agreement.

2. Water vapor

Water vapor, like CO_2, diffuses only out of the animal. We can assume that the tracheal gas is saturated. At 25° C. this means 24 mm. Hg H_2O vapor pressure, or 3% of an atmosphere. During the interburst period of stage III it will diffuse out about half as fast as CO_2 if the external air is dry. When the valves are open in stage I it will flood out like CO_2. Since the wet tissues beyond the tracheae represent a reservoir of water, the vapor pressure value of 3% in the tracheae should be maintained throughout the burst.

Thermal conductivity shows nearly this picture. The curves for CO_2, CO_2 + H_2O, and H_2O alone are shown in Figure 2. A given percentage of H_2O has only half the unbalancing effect on the bridge that CO_2 has. Thus, the combined curve shows a strong excursion in the CO_2 direction at the beginning of a burst.

At the end of the burst there is more H_2O than CO_2 coming out per unit time and the deflection is in the other direction. This indicates that the valves stay open slightly longer than is really necessary. Once a burst has started, pCO_2 no longer directly controls the valves. Or perhaps the triggering mechanism for closure is a slow one.

The surprising feature is that, even in a dry atmosphere, the water vapor lost is on the average about the same amount as the CO_2 produced metabolically. With fat as an energy source (indicated by an R.Q. of 0.7), this is the same as the amount of H_2O produced metabolically. The Cecropia pupa, like the desert rat, can literally produce all the water it needs. In a high relative humidity there should actually be a net gain. Since carbon is being lost, the weight should remain nearly constant. This was tested. Five pupae were kept in a jar over water for four months. They lost an average of only 5% in weight.

If anything happens to the spiracular valves in a dry environment, the insect is doomed. Water loss is now continuous at the burst rate. This means it is at least 25 times greater than before. Three pupae with the spiracular valves cannulated were exposed to 30% relative humidity. In three weeks' time they were 28% to 30% lighter, looked shriveled, and did not appear to be alive. They did not recover when subsequently kept over water.

3. Burst sequence

A large amount of data on burst time sequences has been collected. There is considerable variation in period in a group of identically treated pupae, all in diapause. At 20° C. these intervals varied from 4 to 17 hours. A given individual maintains a remarkably constant cycle for several weeks. A slight lowering of insect environmental temperature shows up as a lengthening of the burst cycle. The triggering CO_2 level of the valves remains constant so the lower metabolic rate takes longer to achieve this value in the tracheae.

When the insect is cooler than about 10°, there appear to be no bursts. Apparently pore diffusion can take care of the low gas exchange rate.

A pupa which has been at the still lower temperature of 6° shows a transiently higher metabolism for one to two days when brought to 25° C. During this time the burst mechanism is inoperative. It recovers slowly over a period of days (Fig. 1a).

In injury metabolism or during development the bursts come closer together and eventually fuse. Slight oscillations show on top of the high value of CO_2 output (Fig. 1b).

Metabolic preparations for the initiation of adult development are clearly indicated by an increase in burst frequency several days before there is any visible evidence of development. The emergence of the adult moth from the pupal skin shows up as a startling increase in CO_2 output which results from the struggle to get out of the respirometer chamber.

Conclusions

New methods have been applied to the dynamics of gas exchange in the tracheal system of pupae of the Cecropia silkworm permitting the direct observation of many features of O_2, CO_2, and N_2 flux that had previously only been deduced from indirect evidence. This system functions so efficiently that water loss is reduced to a value which is approximately the same as that produced metabolically.

LITERATURE CITED

BECKEL, W. E., 1958. The morphology, histology, and physiology of the spiracular regulatory apparatus of *Hyalophora cecropia* (L.). Proceedings of the Tenth Int. Congr. on Entomology. Vol. 2: 87–115.

BUCK, J., 1958. Cyclic CO_2 release in insects. IV. A theory of mechanism. *Biol. Bull.*, 114: 118–140.

BUCK, J., 1962. Some physical aspects of insect respiration. *Ann. Rev. Entomol.*, 7: 27–56.

BUCK, J. B., AND M. L. KEISTER, 1958. Cyclic CO_2 release in diapausing pupae. *J. Ins. Physiol.*, 1: 327–340.

KANWISHER, J., AND A. EBLING, 1957. Composition of swim bladder gases in bathpelagic fishes. *Deep-Sea Res.*, 4: 211–217.

LEVY, R. I., AND H. A. SCHNEIDERMAN, 1958. An experimental solution to the paradox of discontinuous respiration in insects. *Nature*, 182: 491–493.

PUNT, A., 1944. De gaswisseling van enkele bloedzuigende parasieten van warmbloedige dieren (*Cimex, Rhodnius, Triatoma*). *Onderz. Physiol. Lab. Rijks.-Univ. Utrecht, Ser. 8, pt.* **3**: 122–141.

SCHNEIDERMAN, H. A., 1956. Spiracular control of discontinuous respiration in insects. *Nature,* **177**: 1169–1171.

SCHNEIDERMAN, H. A., AND C. M. WILLIAMS, 1955. An experimental analysis of the discontinuous respiration of the Cecropia silkworm. *Biol. Bull.,* **109**: 123–143.

SCHOLANDER, P. F., 1947. Analyzer for accurate estimation of respiratory gases in $\frac{1}{2}$ cc. samples. *J. Biol. Chem.,* **167**: 235–250.

SCHOLANDER, P. F., L. VAN DAM, C. L. CLAFF AND J. KANWISHER, 1955. Microgasometric determination of dissolved O_2 and N_2. *Biol. Bull.,* **109**: 328–334.

WIGGLESWORTH, V. B., 1935. The regulation of respiration in the flea, *Xenopsylla cheopsis,* Roths. *Proc. Roy. Soc. London, Ser. B,* **118**: 397–419.